hydrophilic or hydrophobic material, such as a protein, a suitably hydrophilic or hydrophobic material upon which it may be adsorbed, such as polycarbonate, may be used as substrate **202**. Where interaction materials may be damaged if the membrane dries out after patterning, the substrate may be able to at least temporarily maintain moisture.

[**0092**] Where the substrate is formed as a membrane, it may be desired to support the substrate on a support **208**, for example as illustrated in **FIG. 17**, section c. Support **208** may be constructed in many manner and using any materials that provide sufficient support to substrate **202**. For example, support **208** may be constructed of the same material from which the fluid paths are formed and may be of sufficient size and shape to support the relevant areas of the substrate.

[**0093**] Interaction materials may be patterned onto a substrate in any manner that allows them to applied in the desired pattern and in a manner that makes them accessible for desired interactions. For example, the method may be capable of laying out interaction materials in a pattern to create desired contact points between the interaction materials and the fluid path, and the patterned interaction materials may be accessible to interaction materials in the fluid paths. One suitable method for patterning interaction materials is illustrated in **FIG. 18**. In this method, one or more fluid paths **206** may be placed in fluid contact with substrate **202** and fluid including interaction material therein may be flowed into the fluid path. The interaction material may be allowed to deposit on the substrate, and the fluid removed to immobilize the interaction material, as described previously. The shape of the fluid path or paths may dictate the pattern, both in terms of shape and size, of interaction fluid formed on the substrate. Typically, the shape and size of the interaction material will match the size and shape of the open side of the fluid path from which it is patterned. Additionally, fluid contact need only be made where it is desired to pattern material, and this can be used to further control the patterning of the interaction material. For example, the fluid path may be open to the substrate at intervals, creating spots, dotted lines, or many other patterns, depending on the size and shape of the openings. After the pattern is formed, the fluid paths may be removed from the substrate. Where the patterned interaction material may be relatively sensitive, care may be take to remove the fluid paths such that the interaction material is not substantially damaged, i.e., the interaction fluid will function as desired in a particular embodiment. Additional method of patterning materials on substrates that may be suitable for embodiments of the present invention may be found in PCT Publication No. WO 01/89787, which is hereby incorporated by reference herein in its entirety.

[**0094**] In some embodiments using more than one fluid path may allow different interaction materials to be patterned onto the substrate. The flow paths may be in contact with the substrate at the same time, or different times. In some embodiments, it be desired to form patterns of different interaction materials that overlap. It should be appreciated that embodiments of the method of forming a pattern of material on a substrate of the present invention is not limited to interaction materials and may be used for other purposes, such as the formation of markings, and the like.

[**0095**] In the example embodiment of **FIG. 18**, two fluid paths **206** are used to pattern two stripes of interaction material **200** on a membrane substrate **202**. As shown in **FIG. 17**, section a, the fluid paths are placed in contact with the substrate and the substrate if supported by a support **208**. Fluid including the desired interaction materials (e.g., an aqueous solution of protein) is flowed through the fluid paths as illustrated by flow indicators **100** in **FIG. 17**, section b. The interaction material is deposited on the substrate and the flow paths and support are removed, leaving a substrate patterned with two strips, each including a different interaction material, as illustrated by **FIG. 17**, section c.

[**0096**] Where the reaction to be performed is a test, the results of interactions between fluids in the fluid paths may be observable in any manner. For example, they may be observable visually with the naked eye or with instrumentation, or may be observable by other means, such as through changes in temperature, pH, or the like. Where the reaction is not observable with the naked eye, an instrument, such as a pH meter, optical detection system or thermocouple may be present within or outside the fluid system to make the observation. Even where the reaction is visible to the eye, it may be desired to use an instrument, for example to automate measurement or to measure intensity of fluorescence or absorbance.

[**0097**] In some embodiments, the present invention may use the flow conditions within the fluid paths to further increase the amount of interactions that may be performed. For example, where fluid flows under highly laminar conditions, as is often the case in microfluidic systems, fluid does not mix as it flows, except by diffusion. Accordingly, it is possible to introduce two or more fluids into a fluid path such that they flow in parallel. Such fluid paths containing multiple fluids may effectively act as multiple fluid paths. For example, as illustrated in **FIG. 16**, three fluids, designated **1**, **2** and **3** may flow in fluid path **20** and three fluids designated A, B and C may be flow in fluid path **30**. At contact region **50**, nine different interactions may occur. Techniques suitable for flowing fluids in parallel and the related equipment is described in U.S. Provisional Patent No. 60/286,476, which is hereby incorporated by reference in its entirety.

[**0098**] In certain types of testing, such as immunoassays, it may be desirable to serially dilute test material. One method of serial dilution that may be useful in certain embodiments of the present invention is disclosed PCT Publication No. WO 02/22264, which is hereby incorporated herein by reference in its entirety. Dilution may also be performed according to a method of gradient generation of the present invention. In the method of the present invention, a fluid to be diluted to introduced into a first inlet of a microfluidic device and a dilutant is introduced into a second inlet of the device. The fluid to be diluted then may be split. A first portion of the fluid may pass undiluted into a first flow path, while a second portion of the fluid passes into a second flow path that is also connected to, and receives dilutant from, the second inlet. Accordingly, the fluid may be diluted. A portion of the diluted material may then be passed via a connecting path to a third fluid path while the remainder continues through the second fluid path. The third fluid path may also be connected to the second inlet and the already diluted material may be further diluted. This process may be repeated to achieve as many levels of dilution as desired. This method of dilution allows simultaneous dilution of a material in multiple streams over a wide range of pre-